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EXPERIMENTAL PYELONEPHRITIS IN THE MOUSE

WILLIAM F. KEANE, JR.

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EXPERIMENTAL PYELONEPHRITIS IN THE MOUSE

BY

William F. Keane, Jr.
B. S., Fordham College, 1964

A Thesis

Submitted to the Faculty
of the Yale University School of Medicine
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To My Wife and My Parents

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Introduction

The studies reported in this thesis deal with three closely related aspects of experimental pylonephritis in the mouse. Although there have been other studies of pyelonephritis in the mouse, they have all utilized the hematogenous route for the introduction of bacteria. The experiments to be described have employed direct injections of bacteria into the kidneys and into the bladder thus permitting comparison of data in the mouse with other species more commonly employed for studies of pyelonephritis.

The first section describes the relative susceptibility of the cortex and medulla of the normal kidney to direct inoculation of test bacterial organisms and factors which might influence the course of experimental pyelonephritis in this animal. The second section deals with the effects of water diuresis on bacteriuria. In the concluding section, results are presented demonstrating the effect of water diuresis on the direct inoculation of bacteria into the cortex and medulla.

The materials and methods for all three sections are presented in a separate section preceding the presentation of the three parts of the experimental work.

METHODS and MATERIALS

Animals

Albino male mice Swiss strain (random bred, I.C.R., specific pathogen free, Yale University) weighing 30-40 grams served as experimental animals in all studies. In all experiments the animals were housed in large mouse boxes in groups of six and fed standard Purina laboratory chow.

Bacterial species

E. coli, the organism predominately used in these experiments has been employed in the studies of experimental pyelonephritis in this laboratory for many years and has been previously characterized (1). The inocula for these experiments was derived from a 4½ hour broth culture diluted to achieve the appropriate bacterial concentration.

Staphylococcus Aureus, known as Gorgio, was used in some of the experiments in Part I. An overnight culture diluted with sterile saline to a desired bacterial concentration was used in all instances. This bacteria has been maintained in this laboratory for over seven years, and its characteristics have been described elsewhere (2).

E. coli (Bojko) is an organism obtained from a urine culture of a patient with a long standing urinary tract infection. Further classification of this bacteria by the State Laboratories of Connecticut revealed the following biochemical reactions: Glucose: acid and gas, lactose: acid and gas, maltase: acid, mannitol: acid, rhammose: acid, arabinose: acid, raffinose: acid, xylose: acid. In addition it produced a positive reaction with indole, nitrate and lysine. The inocula employed in some of the experiments in Part I was prepared from a 4½ hour broth culture diluted to the desired bacterial concentration.

Bacteria were enumerated by preparing serial one hundred fold dilutions of the original culture with 0.98 per cent sterile saline. One ml. of these dilutions was then mixed with warm agar and the number of colonies were counted after 24 hours of incubation at 37°c.

Operative technique

Animals were anesthesized with ether and the abdomen shaved and prepared with 70 per cent alcohol or staphene (a disinfectent spray manufactured by Vestal Laboratories, a division of W. R. Grace and Co.). The abdomen was entered through a mid-line incision and depending upon the experiment the bladder or the kidney was then exposed.

Injection of bacteria into the kidney

The left kidney was site of injection in all cases because of easier accessibility. A 27 gauge needle and a syringe calibrated to 1/100 ml. were

used to inject the bacterial suspension. For medullary inoculation 0.03 ml. of the bacterial suspension was used and the needle was inserted perpendicularly to the surface of the kidney approximately one cm. medially from the lateral aspect, midway between the upper and lower poles, and to a depth of 1.82. Omm. The cortex was inoculated by passing the needle tangentially to the lateral aspect a distance of 3-5 mm. approximately 1 mm. beneath the surface of the kidney. 0.5 ml. of the bacterial suspension was slowly injected as the needle was withdrawn. Immediately after removal of the needle from the site of injection slight pressure was applied over the puncture wound to minimize bleeding. The animals were then returned to their cages and allowed to eat and drink freely. At the end of 4-5 days these experiments were terminated.

Injection of bacteria into the urinary bladder

Through a small midline incision the bladder was gently exposed and urine aspirated through a 27 gauge needle. In a second syringe .05 ml of an appropriate dilution of **E. coli** was introduced into the bladder through the same needle. In this way, only one bladder puncture wound was made. The kidney, ureters and urethra were not exposed or manipulated in any way during this procedure. Following recovery from anesthesia the animals were returned to their appropriate cages and allowed to eat and drink **ad libitum.**

Determination of bacteria present in blood, urine and kidney

In all cases .2 ml of a undiluted culture of the test organism was inoculated in the tail vein. At the end of a four day period these animals were then sacrificed.

Determination of bacteria present in blood, urine and kidneys.

After the designated incubation period animals were prepared in the manner described above and sacrificed. Blood was aspirated from the heart and 0.5 ml. to 1 ml. was mixed with warm agar in a pour plate. The bladder was then exposed and urine was aspirated from the bladder and 0.05-.1 ml was then cultured. Both kidneys were carefully removed, placed in sterile petri dishes, sectioned longitudinally and examined for gross lesions. In some instances one half of the kidney was fixed in formalin for microscopic sectioning. The kidneys were then placed in sterile grinding tubes and homogenized in 1.7 ml. of 0.98 per cent sterile saline. When half of the kidneys were cultured 0.85 ml. of 0.98 per cent sterile saline was used. This represents a 1:10 dilution. Subsequent tenfold dilutions were prepared in sterile saline, 1 ml. of the 10^1, 10^3 and in some instances 10^5 dilutions were mixed with warm agar poured into sterile petri dishes. In addition kidney and urine samples were then streaked on blood agar and desoxycholate plates. After incubation at 37°C for 24-36 hours colonies were counted and further identified on Simmon's citrate and Kligler's iron media.

Serum bactericidal studies

Whole blood was sterily aspirated via cardiac puncture from mice, and rabbits. This blood was then carefully placed in a sterile glass centrifuge tube and the clot allowed to retract at room temperature for one hour. Then, with a sterile applicator stick the clot was rimmed and the blood centrifuge for 30 minutes at 2500 RPM. Serum was removed sterilely and stored in a freezer until needed. For all experiments 2 ml. of serum was used. In the case of mice, serum from several mice was pooled in order to achieve this amount. To each 2 ml. of serum was added .02 ml. of a 10^3 dilution of the test organism. .1 ml. aliquots were removed at designated intervals and appropriately diluted with 0.98% sterile saline and 1 ml. of these dilutions was then plated with warm agar. Growth curves of **E. coli** in dilute and concentrated mouse urines were also studied using the method described above. In addition, the ability of a test inoculum of staphyloccus aureus and **E. coli** to grow in a lysozyme extract from rat kidneys was determined in the same way.

Preparation of lysozyme extract

The extraction techniques used in these experiments were developed by Lippmann (3) as a modification of Cohn's and Hirsch's method (4). This involves a homogenization of kidney tissue in hypertonic sucrose, freeze-thawing, and subjecting the tissue to variations of ph, with removal of denatured protein.

Lysozyme determinations

The assay technique used in these experiments was designed by Osserman and Lawlor (5). Unknown samples are placed in wells in an agarose suspension of Micrococcus lysodeikteicus and incubated overnight at room temperature. Known standards were run simultaneously on each plate. The diameter of the ring of lysis around the well created by the unknown was carefully measured. Since the diameter is proportional to the log concentration of lysozyme, through simple mathematical manipulation, the diameter of lysis was converted to ug/ml of lysozyme activity.

Estimation of rental cortical inflammatory response

The renal cortical response to infection was studied in terms of the leukocyte response following injection of **E. coli** into this zone of the left kidney. Following this procedure the animals were returned to their cages awake. At two and five hours following the inoculation, the mice were sacrificed and the left kidney removed, cut perpendicular to the needle tract, fixed in 10% formalin and serially sectioned. Every fifth section was mounted and stained with hematoxylin and eosin and studied microscopically. A minimum of 50 sections from each kidney was studied. The inflammatory response was evaluated by semi-quantitative estimate of the number of leukocytes present at the site of injury.

Experimental Pyelonephritis I. Observations on the susceptability of the mouse kidney to infections by **E. coli and** a virulent strain of Staphylococcus aureus.

Introduction

In man, the majority of cases of pyelonephritis are caused by coliform bacteria. This infection cannot be produced in normal animals even when large numbers of bacteria are inoculated into the blood stream or urinary passages. Virulent strains of staphlococci, however, when inoculated intravenously, are capable of infecting the normal kidney, causing multiple abscesses throughout the renal parenchyma (6,7,8,9).

In 1956 de Navasquez made the important observation that rabbits which had recovered from staphylococcal infection of the kidneys had become susceptible to hematogenous pyelonephritis due to coliform bacteria (10). His studies showed that the bacillary pyogenic infections tended to develop in areas previously damaged by staphylococci, in and around tubules partially obstructed and distened by scars. Further study of this problem by Rocha et. al. indicated that damage to the renal medulla markedly enhanced the susceptibility of the kidney to coliform infection, while injury to the cortex caused no appreciable alteration in susceptibility to hematogenous bacilliary challenge (11). These data suggested a difference in the capacity of these tissue to permit bacterial persistance and multiplication, and subsequently led to the unequivical demonstration of the exquisite susceptibility to infection of the renal medulla (12). Only ten **E. coli** were effective in initiating infection in the renal papilla, whereas nearly a million were necessary to infect the cortex. Further experimentation by Freedman in the rabbit showed that this differential susceptibility also existed when a virulent strain of staphylococci was directly injected into these two zones of the kidney (13). The data presented by these investigators added substantially to the thesis that bacterial multiplication in the kidney took place initially within the medullary tissue.

Recently, it has been suggested by Gorrill et al. that in the mouse the cortex is the initial site of bacterial multiplication in hematogenous staphlococcal pyelonephritis ,14). These conclusions were based on histological demonstration of minute foci of inflammation surrounding colonies of staphylococci within the cortex. This finding is most interesting and suggests that the cortex of the mouse kidney might be more susceptible or at least as susceptible to pyogenic infection as the renal papilla. Furthermore, if this observation is correct, the mouse would be the first animal species in which hematogenous pyleonephritis could be shown to begin in the cortex. Hence, the purpose of this study was to investigate the relative susceptibilities of the kidney cortex and medulla to bactrial infection in the mouse. In addition, experiments were designed to study the role of serum bactericidal factors and lysozyme in the pathogenesis of pyelonephritis in this species.

RESULTS

Intravenous inoculation of Staphylococci and E.-coli in normal mice

To test whether the particular strains of bacteria in these experiments were effective in producing kidney infections, a group of twelve mice were given an intravenous inoculum of 10^7 staphylococci aureus (Gorgio) and approximately 10^8 **E. coli** were given intravenously to fourteen mice. Only two deaths were recorded, both of which occurred on the second day post infection in the group receiving **E. coli.** Neither of these animals demonstrated abscess formation at autopsy and their deaths were ascribed to the effect of endotoxin. By the third day after bacterial challenge, there was a marked difference in appearance between the two groups. The mice given staphylococci became less active and were observed in a hunched position in a corner of the cages. In addition, they appeared to eat little and drink only small amounts of water. At the end of a four day period both groups were sacrified and as can be seen in table I all of the animals given staphylococci had pyelonephritis. These results were comparable to those achived by other investigators (2,7).

In contrast, mice given **E. coli** had no macroscopic lesions at necropsy and there was no evidence of infection when kidney homogenetes and urine were cultured. These results are consistent with those in normal rats and rabbits given **E. coli** intravenously.

The **E. coli** used in this experiment is a relatively a virulent organism and has been maintained in this laboratory for over ten years. To test the possibility that a more virulent organism might influence the rate of infection, colonies of **E. coli** (Bojko) freshly isolated from a patient with severe long standing urinary tract infection were employed. These organisms however, were unable to induce pyelonephritis in 14 mice when 10^8 **E. coli** were inoculated via the intravenous route. (Table II). Five of the 14 mice challenged with this **E. coli** died within 24 hours without evidence of kidney infection.

Kidney lesions produced by intravenous inoculation of staphylococci

The macroscopic lesions found in infected kidneys were characterized by formation of abscesses in the cortical and medullary regions. Abscesses in the cortical regions were rarely larger than 1mm. in diameter and generally showed extension into the medullary zone. In contrast, the lesions which involved the medulla were larger, 2-3 mm. in diameters and extended into the papilla. In a few instances there was extension into the cortex. There was no evidence of papillary necrosis, nor was there any evidence of obstruction of the lower urinary tract.

Direct inoculation of bacteria into different zones of the kidney

To investigate the susceptibility of the kidney to the test organisms, serial one hundred fold dilutions of **E. coli** and staphylococci were directly injected into the cortical and medullary zones. Animals were sacrified four or five days after injection of bacteria. These findings are presented in tables III and IV.

Although 10^3 staphylococci are required to establish infection when placed directly in the cortex of the kidney, the inoculation of as few as 10 bacteria into the medulla resulted in the production of pyelonephritis. The abscesses produced by cortical inoculation were usually localized to the area of injection and did not show any evidence of extension into the papilla. However, when very large numbers of the test organism were used the infection often involved both the cortex and the medulla. The infection produced by medullary inoculation consisted of a collection of small abscesses in a wedge shaped area, with the apex in the papilla and the base in the cortex. The cortical region involved seemed to be tributaries to the collecting tubules passing through the site of injection. The area of involvement was sharply demarcated from the surrounding tissue, without any obvious lateral extension in either cortex or medulla. Despite the profusion of bacteria and pus cells in the kidney pelvis, infection was detected only in areas affected by original injury. This is remarkable in view of the capacity ofstaphylococci to infect the normal kidney via the blood stream. One might have anticipated the same infectivity to be apparent by either direct extension of infection through the kidney substance, or by bathing of normal papillary tissue in heavily infected urine. Finally, although large numbers of bacteria were occassionally recovered on culture of the contralateral kidney, there was no evidence of abscess formation.

When **E. coli** were injected into the cortex and papilla of the mouse kidney results similar to those found with staphylococci as the test organism were obtained. As with the abscesses produced with staphylococci, the macroscepisc lesions seen were confined to the injection site and only when a large inoculum was used was there evidence of extension beyond this region. Significant numbers of bacteria recovered from the opposite kidney were not associated with any gross lesion. These experiments in the mouse, as in the rabbit demonstrate that the same number of **E. coli** and straphylococci are required to produce infection in the kidney. This is a remarkable finding since intravenous **E. coli** do not produce pyelonephritis in the normal kidney, while hematogenous pyelonephritis is readily induced by staphylococci. This would seem to indicate that the factors most decisive in determining virulence for these two organisms is the effectiveness of host defense mechanisms as they operate in the circulating blood.

Serum bactericidal activity

It was of interest that the number of bacteria necessary to infect the cortex of the mouse kidney was significantly less than in the rabbit. Since Mac Leod et. al. have shown a correlation between serum antibacterial activity and the production of subcutaneous abscesses in the mouse and rabbit (15), experiments were designed to test the possibility that the decreased cortical susceptibility to pyelonephritis in the mouse might be related to the differences in serum bactericidal activity. In mouse serum following a lag period of approximately two hours rapid growth of staphylococci paralleled that seen in nutrient broth. In contrast, rabbit serum showed consistent and marked bactericidal activity. Decrease in the number of staphylococci occurred rapidly and within six hours the number of viable units on colony count was less than one hundred (Fig. II). When a similar inoculum of **E.coli** was tested, botr rabbit and mouse sera adequately supported the growth of this bacteria (Fig. I). Hence, although there is a correlation between antistaphylococcal activity of mouse and rabbit sera and the production of cortical abscesses, the absence of any differences in the serum antibacterial activity for **E. coli** in these animals and the fact that similar numbers of **E. coli** and staphylococci can infect the cortex suggests that factors other than those determining serum bactericidal activity are necessary to explain why fewer organism are necessary to infect the cortex of the kidney in the mouse as compared to the rabbit.

Kidney Lysozyme

Since the papilla of the rat, rabbit and mouse is more susceptible to **E.coli** and staphylococcal pyelonephritis than the cortex, it is natural to look for some antibacterial substance that is present in the cortex but absent or greatly reduced in the medulla as a possible explanation for this phenomenon. Since the kidney has an extremely high titre of lyscozyme (16) an important lytic enzyme which perhaps is involved in host defense mechanisms, experiments were designed to measure the concentration of this enzyme in the cortex and medulla of the rat and mouse and to determine its bactericidal potency against the test organisms.

Recent investigations using histochemical analysis have indicated that lysozyme is largely located in the proximal tubules of the kidney (17, 18). These findings suggested that the concentration of lysozyme in the cortex might be significantly higher than in the medulla. To test this hypothesis, the cortex and medulla of eight rat kidneys and six mouse kidneys were carefully separated and the relative concentrations of lysozyme determined in each part. (Table V). These studies clearly demonstrated that the lysozyme content of the renal cortex was two to three times as great as that of the medulla.

Subsequently, studies designed to establish the in vitro sensativity of **E.coli** and staphylococci to rat kidney lysozyme, demonstrated that 600 ug/ml of lysozyme was not effective in preventing the growth of **E.coli** whereas less than 10 ug/ml of lysozyme rapidly destroyed this strain of staphylococci. It is interesting to note that although less than 10 ug/ml of this enzyme are needed to destroy over 10^3 staphylococci, a similar number of viable units will cause pyelonephritis when directly injected into the mouse kidney cortex, a zone containing nearly a hundred times the amount of lysozyme necessary to lyse this number of bacteria in vitro. Therefore, it would appear that kidney lysozyne is not one of those factors which determine susceptibility to infection in the kidney.

Discussion

These experiments confirm earlier studies in the rabbit which demonstrated that the medulla of the kidney is more susceptible to pyogenic infection than the renal cortex. Whereas 10^3 staphylococci or **E. coli** produce pyelonephritis when directly injected into the renal cortex of the mouse, fewer than 10 bacteria will infect the medulla.

The intravenous injection of staphylococci resulting in acute pyelonephritis confirms the work of other investigators. Although Gorrill et. al. observed that inflammatory foci first originated in the glomeruli and therefore lead to the formation of cortical abscesses. (8), it is probably not possible to judge the behavior of bacteria in tissues by observing the inflammatory response they provoke. This point is of crucial importance in the kidney, since estimations of the inflammatory response both to thermal injury and bacterial inoculation in the medulla of the rat and rabbit kidney is markedly delayed and diminished in intensity when compared with the response of the cortex (19, 20). Furthermore, the fact that inflammatory response was not seen in the medulla, but in the cortex would correlate well with the findings of the present studies demonstrating the exquisite sensitivity of the medulla to pylonephritis following direct injection of bacteria into this region. Indeed adequate and rapid leukocyte response has been implicated by many observers as one of the major determinants of infection. Therefore, it is most probable that the inflammatory foci seen within the cortex and not in the medulla in Gorrill's et. al. experiments can be explained by the enhanced leukocyte response present in the cortex as compared to the medulla. Although these studies do not conclusively eliminate the possibility that bacteria multiplication occurred initially in the cortex, they make it appear unlikely. The most direct way of resolving this issue would be by culturing the cortex and papilla separately at intervals following intravenous injection of the test organism.

Despite the difference in the ability of **E. coli** and staphylococci to produce infection by the hematogenous route, the present studies have

demonstrated that similar numbers of these bacteria are required to produce infection of different zones of the kidney when they are injected directly. Infection is produced with as few as 10 organisms in the medulla, whereas more than 1000 are required to infect the cortex. The lesions produced by direct inoculation of either of these organisms are similar in appearance. While one would expect to find significant differences in the pathogenicity of these organisms, judging by their effects when given intravenously, these studies have confirmed experiments in the rabbit demonstarting the remarkable similarities in their ability to produce infections when directly injected into the kidney. One possibility which has been suggested to explain the differences in virulence of these two organisms when given intravenously is the effectiveness of host defense mechanisms as they operate in circulating blood (13). Cohn and Morse have provided data which support this thesis by demonstrating that **E. coli** are more vulnerable to phagocytosis and destruction by leukocytes than are virulent staphylococci when these organisms are incubated with normal rabbit leukocytes (21).

An interesting finding in these experiments was the number of viable organisms necessary to infect the cortex of the mouse. This was consistently 2-3 logs lower than that required in either rat or rabbit. One explanation that was considered was the relative smaller size of the mouse kidney. It is conceivable that when the bacteria was injected in the cortex, some could have been dissapated within the medullary tissue and thereby initiating infection with fewer bacteria. However, this does not seem to be the sole answer since the lesions formed when 10^3 - 10^4 organisms were inoculated were confined only to the site of injection.

Another explanation that was suggested by experiments in Part III of these studies is the number of bacteria recoverable from the mouse kidney immediately following direct injection of bacteria into the cortex. These experiments indicated that after 60 minutes the number of bacteria recoverable from the inoculated kidney was equal to the number injected initially. This is in contrast to the rabbit where after only five minutes the number of bacteria recoverable is reduced by as much as 100 fold (12). Hence, one possible explanation for the greater susceptibility of the mouse cortex is the decreased immediate clearance of bacteria from this region. Although the explanation for this difference between the mouse and rabbit is not known, it may be related to differences in renal cortical blood flow in these animals.

An attempt was made to investigate the effect of certain host defense mechanisms in the pathogenesis of pyelonephritis. The first factor that was studied was the antibacterial activity of rabbit and mouse serum. These studies showed that rabbit serum is bactericidal to staphylococci and mouse serum is not. However, neither serum demonstrated antibacterial activity against **E. coli.** The differences in serum anti-staphylococcal activity of

these animals corresponds to the relatively fewer staphylococci necessary to infect the renal cortex of the mouse. On the other hand, equal numbers of staphlococci and **E. coli** will infect the cortex of the rabbit despite the marked differences in bactericidal activity against these two organisms. Hence, from these in vitro studies of antibacterial activity of rabbit and mouse serum it would appear that this factor assumes a relatively minor role, if any, in the pathogenesis of renal cortical infections. Since complement has been demonstrated to be important for the operation of certain antibacterial factors, one explanation for this apparent lack of activity of bactericidins within the kidney is perhaps related to the anti-complementary activity of renal tissue (22).

The second host defense factor that was investigated was lysozyme or muramidase. This substance was extremely effective in lysing staphylococci but relatively unsuccessful in even inhibiting the growth of **E. coli** in-vitro. This preferential activity of lysozyme is related to its ability to split the glycosidic bonds of amino sugars, which are a major constituent of the cell wall of gram-positive organisms but a relatively minor component of the cell walls of many gram-negative bacteria (23). The kidney contains extremely high concentrations of this enzyme and the present studies indicate that its concentration within the cortex is two to three times that found in the medulla. The precise function of lysozyme in the kidney, however, remains unknown. Unlike other lysozomal enzymes it does not appear to be available to act on its natural substrate. Following direct inoculation of bacteria into the renal cortex of rats, there is a generalized increase in lysozyme content of the serum and both kidneys but no specific increase in the inoculated kidney (24). This generalized lysozmal response to infection has also been noted in the mouse and ascribed to the result of stimulation of th hosts reticulo-endothelial systm (25). Furthermore, the present in vitro studies suggest although kidney lysozyme is an extremely potent antibacterial agent it does not seem to play a major role in host defense mechanisms in the kidney through its ability to lyse gram-positive micro-organisms. This is supported by the finding that the concentration of lysozyme in the cortex and medulla of the mouse and rat is many times greater than that required to lyse staphylococci in vitro, and yet similar inocula of **E. coli** and staphylococci will produce pyelonephritis when directly injected into the kidney.

SUMMARY

Observations were made on the relative susceptibility of the mouse renal cortex and medulla to infection by inoculating coliform bacilli and staphylococci into each zone.

Infection resulted from the introduction of fewer than 10 bacteria in the medulla, whereas over 1000 were usually required to establish infection in the cortex. The lesion which developed following cortical injection is an abscess usually localized to the site of injection. With medullary inoculation, the area of infection is wedge-shaped, involving both medullary and cortical tissue.

Renal infection by the intravenous route in normal mice was produced with 10^7 staphylococci, however no infection could be documented when as many as 10^8 **E. coli** were introduced by the hematogenous route. Aside from this one feature, however, the present studies based on direct inoculation of bacteria into the kidney emphasize the remarkable similarities in the capacity of these organism to produce infection.

Finally, it was not possible to correlate susceptibility to infection in the kidney with either serum bactericidal activity or the antibacterial effect of kidney lysozyme.

APPENDIX I

Table I: Intravenous inoculation of bacteria into normal mice. Bacteriologic studies after 4 - days. Kidneys underlined were observed to have gross abscesses.

10^7 Staphylococci			10^8 E. coli***		
Lt. Kidney*	Rt. Kidney*	Urine**	Lt. Kidney*	Rt. Kidney*	Urine**
10^3	10^5	10^4	0	0	0
10^6	10^4	10^5	0	0	0
10^3	10^5	10^4	0	10^3	0
10^6	10^4	10^5	0	0	0
10^6	10^6	10^5	0	0	0
10^4	10^3	10^4	10^2	0	0
10^5	10^2	10^5	0	0	0
10^3	10^5	10^3	10^3	0	10
10^6	10^3	10^5	0	0	0
10^5	10^4	10^5	0	0	0

*Colonies per kidney.

**Colonies per ml.

***Two animals died in this group and they are not included in these results.

Table II: Intravenous inoculation of 10^8 E. coli. (Bojko) into normal mice. Bacteriologic studies after four days.***

Lt. Kidney*	Rt. Kidney*	Urine**
0	0	0
0	0	0
0	10^2	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

* Colonies per kidney.

** Colonies per ml.

*** The five animals that died before the four day period ended are not included in these results.

Table III: Direct injection of bacteria into cortex of the left kidney of the mouse: Bacteriologic studies after 4 - 5 days. Kidneys underlined were observed to have gross abscesses. Mice drinking tap water.

	E. coli			Staphylococci		
Inoculum	Lt. Kidney*	Rt. Kidney*	Urine**	Lt. Kidney*	Rt. Kidney*	Urine**
10^6	10^6	10^2	10^5	10^7	10^3	10^5
	10^6	10^3	10^5	10^6	10	10^6
	10^7	10^2	10^6	10^7	10^2	10^5
	10^7	10^2	10^6	10^7	10	10^5
	10^6	10^4	10^5	10^7	10^2	10^6
	10^7	10^2	10^5			
	10^7	10	10^5			
10^5	10^6	10^3	10^5	10^7	10	10^5
	10^7	10^2	10^6	10^6	10^2	10^5
	10^6	10	10^5	10^6	10	10^5
	10^7	10	10^5	10^7	10^2	10^6
	10^6	10^2	10^5	10^6	10	10^5
	10^6	10	10^5			
10^4	10^6	10^2	10^5	10^6	0	10^5
	10^5	10	10^5	10^7	10^2	10^6
	10^7	10^3	10^6	10^6	0	10^5
	10^6	10^2	10^4	10^6	10	10^5
	10^6	10^2	10^5	10^6	10	10^5
	10^6	10^2	10^4			
10^3	10^5	10^2	10^4	10^7	10^2	10^6
	10^3	0	10^3	0	0	0
	10^3	0	10^2	10^7	10	10^5
	10^2	0	10^3	10^6	0	10^5
	10^6	10	10^5	0	0	0
	0	0	0	10^2	0	10^2
10^2	10^2	0	10^2	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
10	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0			

* Colonies per kidney.
** Colonies per ml.

Table IV: Direct injection of bacteria into papilla of the left kidney of the mouse: Bacteriologic studies after 4 - 5 days. Kidneys underlined were observed to have gross abscesses. Mice drinking tap water.

	E. coli			Staphylococci		
Inoculum	Lt. Kidney*	Rt. Kidney*	Urine**	Lt. Kidney*	Rt. Kidney*	Urine**
10^6	$\underline{10^7}$	10^2	10^5	$\underline{10^6}$	10	10^6
	$\underline{10^7}$	10^2	10^6	$\underline{10^7}$	10^2	10^6
	$\underline{10^7}$	10^3	10^5	$\underline{10^7}$	10^2	10^5
10^5	$\underline{10^6}$	10	10^6	$\underline{10^7}$	10^2	10^6
	$\underline{10^7}$	10^3	10^6	$\underline{10^7}$	10	10^5
	$\underline{10^6}$	10^2	10^5	$\underline{10^7}$	10^3	10^5
10^4	$\underline{10^6}$	10^2	10^5	$\underline{10^5}$	10^2	10^4
	$\underline{10^7}$	10^3	10^5	$\underline{10^4}$	0	10^3
	$\underline{10^6}$	10	10^4	$\underline{10^5}$	10^2	10^4
	$\underline{10^6}$	10	10^5	$\underline{10^6}$	10^3	10^5
10^3	$\underline{10^5}$	0	10^4	$\underline{10^6}$	10^2	10^5
	10^3	0	10^3	10^3	0	10^2
	$\underline{10^5}$	10	10^4	$\underline{10^5}$	10^2	10^4
	$\underline{10^6}$	10^2	10^5	$\underline{10^6}$	10^2	10^5
	10^3	0	10^2	$\underline{10^6}$	10	10^4
	0	0	0			
10^2	10	0	0	0	0	0
	10^4	10	10^3	10^3	0	10^2
	0	0	0	$\underline{10^5}$	10^2	10^4
	$\underline{10^6}$	10	10^5	$\underline{10^6}$	10^2	10^4
	$\underline{10^5}$	10^2	10^4	10^2	0	10^2
	10^3	0	10^2	10^6	10^2	10^5
	0	0	0	$\underline{10^7}$	10^2	10^5
				10^2	0	10^2
10	10^2	0	10	0	0	0
	10^3	0	10^3	10^2	0	10^3
	$\underline{10^5}$	10	10^4	$\underline{10^5}$	10	10^4
	10	0	0	0	0	0
	0	0	0	10^4	0	10^3
	0	0	0	$\underline{10^5}$	10^2	10^5
	10^3	10^2	10^3	$\underline{10^5}$	10^2	10^4
	0	0	0	10	0	0
	$\underline{10^5}$	10^3	10^4			

* Colonies per kidney.
** Colonies per ml.

Table V: Concentration of lysozyme in cortex and medulla of normal rat and mouse kidneys. These figures represent the average of 8 cortical and 8 medullary samples from the rat and 6 cortical and 6 medullary samples from the mouse.

Rat Kidney		Mouse Kidney	
Cortex*	Medulla*	Cortex*	Medulla*
1700 ± 250	850 ± 145	750 ± 125	275 ± 65

* mg/gram tissue

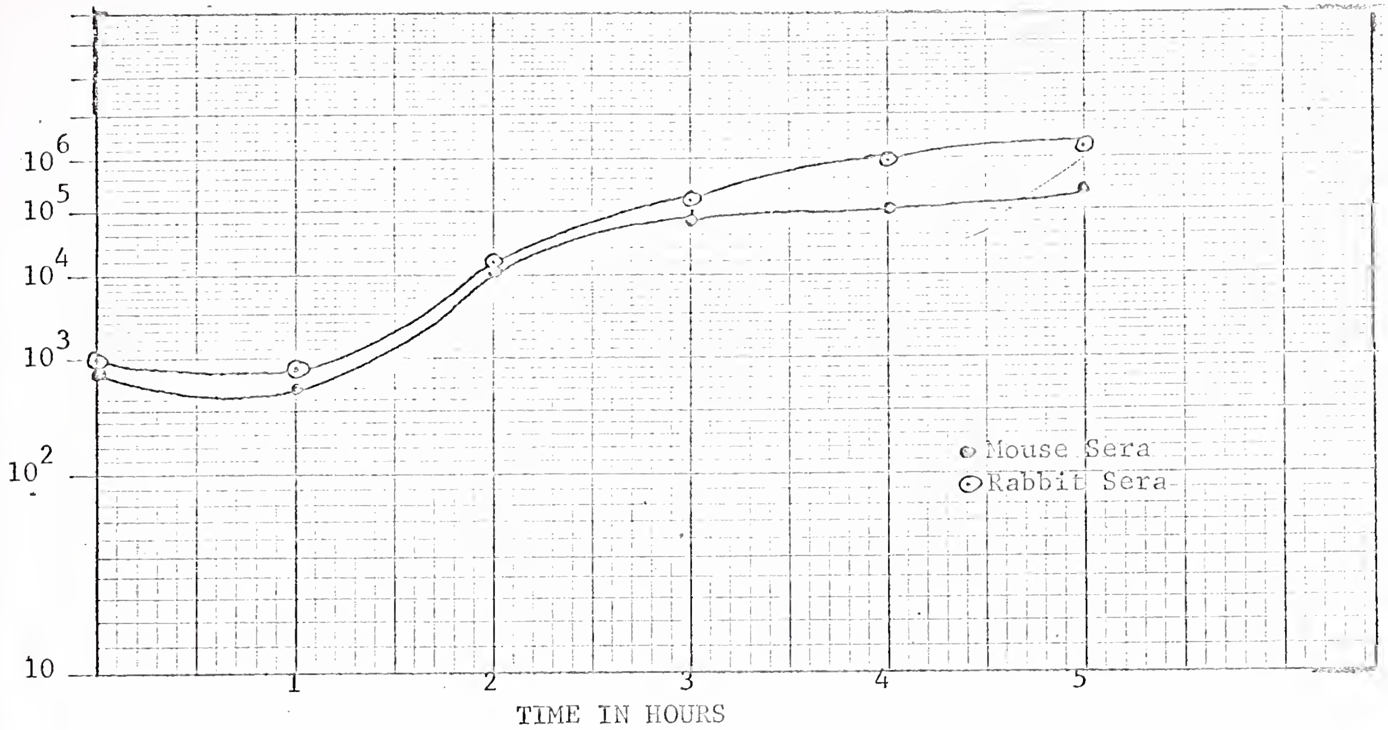

Figure I : Growth of E. coli in rabbit and mouse sera.

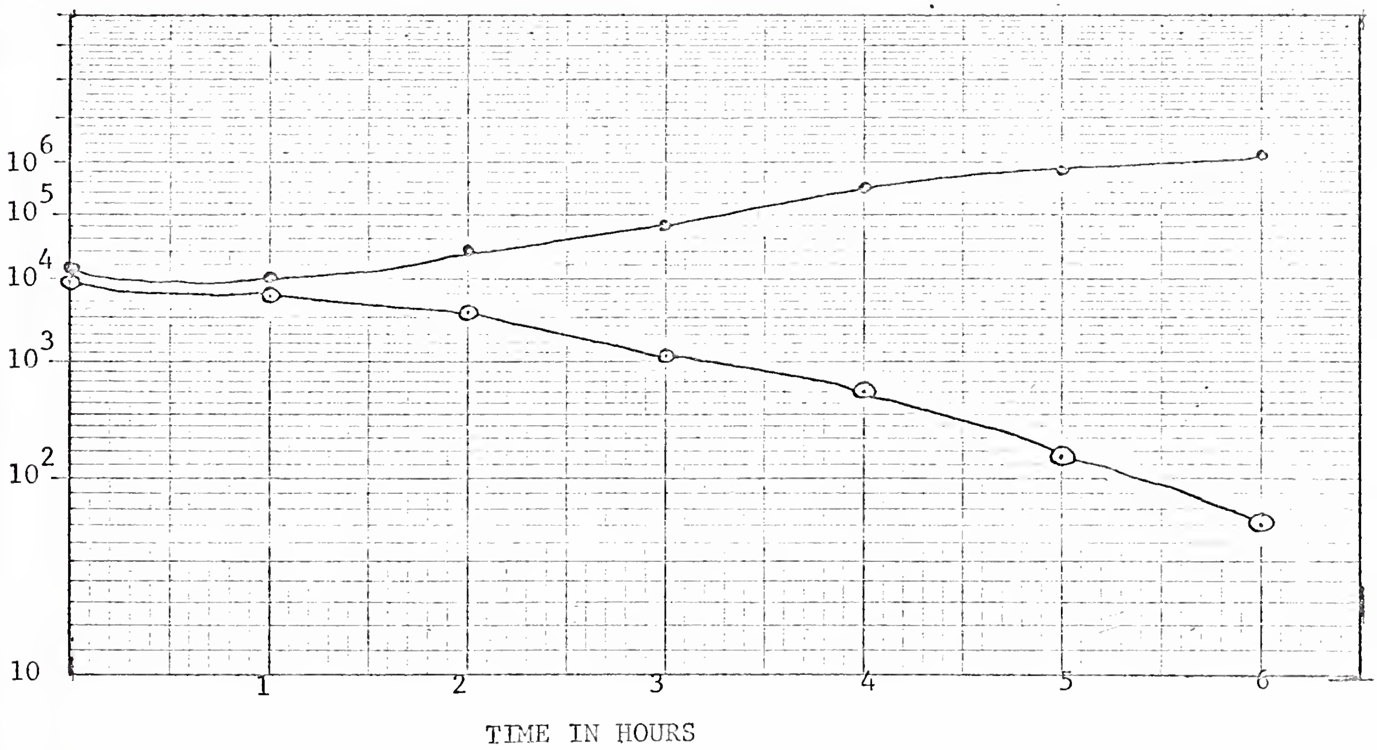

Figure II : Growth of staphylococci in rabbit and mouse sera.

Experimental Pyelonephritis II. Pyelonephritis in normal mice produced by inoculation of **E.coli** into bladder lumen during water diuresis*

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*Presented in part at the Eastern section meeting of the American Federation for Clinical Research and published in abstract form (26).

Introduction

The present body of experimental evidence is strongly suggestive that the principal mechanism by which bacteria reach the kidney producing pyelonephritis is by ascending from the urinary bladder to the kidney via the ureters (27, 28, 29). Although there has been a good deal of study devoted to the factors involved in the susceptability of the kidney to bacterial infection, relatively few data have been published concerning the fate of bacteria in the urine and the importance of this in the pathogenesis of pyelonephritis.

Bladder urine is normally sterile and large numbers of bacteria are rapidly and efficiently cleared from the bladder lumen of normal men and animals (30, 31). Freedman, using a model of rats undergoing a water diuresis clearly demonstrated that as few as 10 **E. coli** may rapidly multiply and persist in the urinary tract for prolonged periods of time (30). Although in the rat large numbers of bacteria persisted and were recoverable for up to 8 weeks, there was no gross evidence of renal parenchymal infection.

The present studies were undertaken to test whether the same phenomenon was demonstrable in mice and, if so, whether substantial renal damage was more likely to result from exposure of the kidney to the large numbers of bacteria in the urine.

Experimental design

Experiments were always carried out simultaneously in two groups of mice, one drinking autoclaved tap water and the other drinking 5% glucose solution. These studies were carried out in both groups for two different time periods, one terminating at the end of one week and the other at the end of 3 weeks. The urine glucose was tested with uristixs and the blood glucose was determined in the hospital laboratory by the glucose oxidase method (46).

Result

Chronic water diuresis

A chronic water diuresis was induced by permitting mice free access to 5 per cent glucose as drinking water. The controls were given free access to tap water. The animals were caged in groups of six and always started on the glucose solutions three of four days preoperatively. The amount of 5 per cent glucose consumed by the mice was 2-3 times as much fluid as those drinking tap water. The urine of mice drinking 5 percent glucose regularly had a dilute urine with specific gravities less than 1.020 in contrast to the rather concentrated urine of mice drinking tap water. There was no evidence of glycosuria in the mice drinking 5 per cent glucose and the nonfasting blood glucose levels while drinking 5 per cent glucose on the average were the same as in animals drinking tap water. A summary of this data is presented in table I.

Effect of water diuresis on clearance of bacteria from the bladder urine

The first series of experiments was designed to determine the effect of water diuresis on the clearance of bacteria from the bladder urine. Water diuresis was established by permitting a group of mice free access to 5% glucose for 3-4 days. At the end of this period, 10^3 or 10^4 **E.coli** were introduced into the urinary bladder in mice undergoing water diuresis and in controls drinking tap water. Both groups were maintained on their different drinking solutions for 4 additional days at which time bacteriological studies were undertaken.

Cultures demonstrated large numbers of bacteria in the urine and kidneys of animals drinking 5 percent glucose solution, whereas bacteria were only rarely recovered from the urine and kidneys of animals drinking tap water (Table II). There was no gross evidence of renal infection in any of the mice. In those instances where bacteria were recovered from animals drinking tap water the total number of viable bacterial units per kidney or per ml. of urine never exceeded 10. Blood cultures were sterile in all instance. At the time of bacteriological study, the bladders of mice drinking glucose were sometimes distended up to a volume of 1.0 cc. However, no evidence of hydroureter or hydronephrosis was observed.

Effect of chronic bacteriuria on the kidney

Since large numbers of bacteria were recovered from the kidneys and urine without gross evidence of infection in mice drinking 5 percent glucose for only four days, it was decided to determine the effect on the kidney after a longer period of bacteriuria. Nineteen mice were continued on 5 percent glucose drinking water over a three week period following the introduction of 10^4 **E.coli** into the bladder urine. A similar number of **E. coli** were injected into the bladder of eighteen mice drinking plain water. Nine of the nineteen mice drinking 5 percent glucose had evidence of urine infection (Table III).

In 8 animals, 10 kidneys had multiple abscesses easily visible on the surface of the kidney (Fig. I). The abscesses occupied the greatest area in the cortex and had the usual wedge shape extending into the papilla. The lesions were segmental, alternating with zones of normal appearing kidney. The intervening zones of normal renal tissue were substantiated on microscopic study of tissue sections.

Papillary necrosis was an additional finding in 4 of the 10 kidneys (Fig. II). In one instance the papillary necrosis involved only a portion of the papilla. There was no evidence of extrarenal obstruction in any of the animals. Histological examination of the bladder revealed no evidence of cystitis.

Bacterial contamination of the drinking water, hydronephrosis and renal damage in mice drinking 5% glucose

In considering possible explanations for the increased susceptibility to infection in animals drinking 5% glucose, the first that was examined was the role of bacterial contamination of the drinking water. Bacterial growth in 5% glucose drinking bottles had been demonstrated previosuly (30). The 5% glucose drinking water was intentionally contaminated with 1.0 ml. of nutrient broth containing 10^8 **E.coli** per bottle of 500 cc. Thirteen mice were allowed access to this contaminated 5% glucose for 4 days and then had sterile saline injected into the lumen of the bladder. After an additional 21 days, during which time the mice received only contaminated drinking water, cultures of the kidneys and urine were sterile. Thus, it was evident that bacterial contamination of the 5% glucose drinking water could not, in conjunction with a sham operation, account for the production of urinary infections.

The bladders of mice drinking 5% glucose were frequently distended. Although hydroureter and hydronephrosis were never observed, the possibility existed, nevertheless, that distention of the ureters or renal pelvis might be present and yet not be obvious at laporotomy. Consequently, intravenously pyelograms were obtained in 3 normal mice, 3 mice drinking 5% glucose for 21 days and 6 mice drinking 5% glucose who were inoculated with **E.coli** into the bladder lumen and examined 21 days later. In the 6 mice drinking 5% glucose, 5 had positive urine cultures and 3 had single kidneys with gross evidence of pyelonephritis. In 2 of the 3 instances where gross abscesses were observed the kidney was not viualized by pyelography. However, the opposite kidney of these animals and all kidneys in the others appeared normal, without distention of the renal pelves or ureters. Examination of the urinary system of these animals immediately after the x-rays were taken confirmed the absence of any evidence of hydronephrosis or hydroureter. Thus, although hydronephrosis and hydroureter were not observed in animals without pyelonephritis, it is not possible to definitely exclude some abnormality of ureteral function on the side of the kidneys with gross abscesses. The evidence, however, would appear to be against this possibility.

An additional experiment was carried out to test whether drinking 5% glucose might have induced some increase in renal susceptibility to infection. This possibility was examined by testing susceptibility to pyelonephritis after intravenous inoculation of 10^8 **E.coli** in 9 mice drinking 5% glucose for 7 days and in 9 control mice drinking water. After four days, bacteriological cultures of the urine and both kidneys were sterile (Table IV). Thus, as measured by susptibility to infection following intravenous challenge, drinking 5% glucose does not increase renal susceptibility to infection.

Growth of bacteria in urine

Another factor that was studied in an attempt to elucidate the pathogenesis of these infections was the ability of this bacteria to grow in dilute and concentrated urine. Figure II demonstrates that bacteria grow more rapidly in dilute urine, parelleling that seen in nutrient broth. On the other hand, in urines of mice drinking tap water the bacterial growth is slower and the number of viable colonies present after a specific interval of time is always significantly less than that found in the dilute urine of mice drinking glucose water.

Discussion

These experiments confirm similar studies in the rat which demonstrated a remarkable decrease in the ability of animals undergoing water diuresis to clear **E. coli** from the bladder urine (30). In previous experiments on rats, kidney infection was not evident after eight weeks of continuous bacteriuria, during which time it was likely that large number of bacteria were frequently in contact with the renal pelvis. In the mouse, however, large numbers of bacteria in the urine in association with water diuresis results in sever pyelonephritis. It is important to emphasize that the microorganism used in the present experiments has been maintained in this laboratory for over 10 years and is relatively avirulent. It has never produced pyelonephritis by intravenous inoculation in normal animals, nor was it able to cause infection when given intravenously to animals with potassium deficiency (32).

The pathogensis of pyelonephritis in the present studies is not precisely known. However, there are certain effects of water diuresis which may be important in these studies. The in vitro experiments using mouse urines have indicated that **E. coli** grow more rapidly in the dilute urine of mice undergoing a water diuresis than in the urines of mice drinking tap water. In addition, the greater distention of the urinary bladder seen in mice drinking 5 percent glucose lessens the opportunity for phagocytosis (33) and impedes blood flow to the bladder musoca (34) resulting in a diminished or altered inflammatory response. It is also possible that high rates of urine production act by providing a constant pool of bladder urine in much the same way as residual urine in patients with partial obstruction to urine flow. Increased urine flow might alter other as yet unknown factors such as opsonic substances in the urine which may be diluted to the point of ineffectiveness.

Another contributing factor in the pathogenesis of pyelonephritis in this report is probably related to the ease with which vesico-ureteral reflux occurs in small animals. Reflux of urine from the bladder up the ureters is well known in rats and the unusual vulnerability of the fornices has been demonstrated by others (35, 36, 37). In studies of the pathogenesis of ascending pyelonephritis of others, the bacterial inoculum employed contained

10^3-10^8 organisms and a large enough volume of culture was infused through a urethral catheter to insure reflux of the full strength inoculum up the ureters. In addition, it has been found thaat urethral catherization in the rat combined with procedures to insure ureteral reflux can result in "spontaneous" kidney infection with organisms normally residing in the urethers (38).

On the other hand, the production pyelonephritis in the present experiments does not involve urethral catherization and is accomplished with inocula that are likely to be encountered clinically. In addition, if reflux from the bladder up the ureters is an important factor in the present studies, it must not be the only factor since within 4-5 days pyelonephritis is not evident despite the recovery of large numbers of bacteria from cultures of the kidneys. This suggests that prolonged bacteriuria might bring about changes in the urinary tract which are a prerequisite to significant kidney infection. It is not clear what these changes are. For example, inflammation and edema of the ureter might result in transient obstruction. However, neither intravenous pyelograms nor gross inspection revealed evidence of hydroureter or hydronephrosis. Nevertheless, transient obstruction might initiate infection and not be obvious by either of these techniques. If transient ureteral obstruction is the explanation for the development of pyelonephritis in the mouse, the large diameter of the ureter in the rat might offer greater protection against the effects of local inflammation and might be why pyelonephritis has not been observed in this species.

In considering other explanations for the effect of water diuresis a possible systemic role of the large amount of glucose consumed is difficult to eliminate. In this regard, it is important to point out that drinking 5 percent glucose was not associated with elevated glucose levels or glycosuria. In addition, data were presented which rule out kidney damage as a possible factor since animals drinking large quantities of glucose solutions were, as normal animals, not infected after intravenous challenge with 10^8 **E. coli.** Finally, although the animal's drinking solutions were changed daily, the possibility that contamination of these by gram-negative organisms might be playing a role in these infections was excluded.

Whatever the mechanism involved in the production of pyelonephritis in this study, it would seem that there is a delicate balance of factors which determine the outcome of bacterial multiplication within the bladder lumen. The outstanding finding in these studies was the extent to which it was possible to tip the balance in normal animals in favor of the bacteria by merely increasing water intake.

In contrast to the experiments reported, there are convincing data which demonstrate the beneficial effects of water diuresis on certain types of pyelonephritis in animals. These studies were concerned with hematogenous infections with staphylocci or enterococci (39, 40) or proteus infec-

tion in the rat, a model known to be associated with a high rate of stone formation (41). Since different mechanisms of infection are involved, the data from these studies are not in conflict with those described in the present report. The issue as to which animal model has the greatest significance for man must await further studies of mechanisms. For the moment, however, the model described in this report, gram negative rod infection in the bladder urine followed by pyelonephritis, seems close to the events as they have been observed in most human urinary infections (42) and leads one to question the value of the common practice of increasing fluid intake in the treatment of urinary infections in man.

Summary:

When **E. coli** are inoculated into the bladder urine of normal mice they are cleared rapidly. If, however, the mice are undergoing water diuresis, bacteria are able to multiply and persist in the bladder urine and produce severe pyelonephritis and papillary necrosis.

Although large numbers of bacteria are in contact with the kidney pelvis during the period of bacteriuria, pyelonephritis did not occur during the first 4-5 days of bladder urine infection. This suggests that chronic bacteriuria may produce changes within the urinary tract that are a prerequisite for the development of pyelonephritis.

Although there are many possibilities that may be invoked to explain these results, it is not known which mechanisms are critical. However, it was possible to rule out factors such as glycosuria, hyperglycemia, hydronephrosis and kidney damage as having significant roles.

APPENDIX II

TABLE I

	TAP WATER	5 % GLUCOSE
FLUID INTAKE / 24° / MOUSE	8 - 12 cc	18 - 27 cc
URINE SPECIFIC GRAVITY	>1.045	<1.020
GLYCOSURIA	Neg.	Neg.
BLOOD GLUCOSE LEVELS (11 mice in each group)		
RANGE	98 - 187 mg %	100 - 173 mg %
AVG.	126 mg %	127 mg %

TABLE II: Direct injection of *E.coli* into bladder of mice drinking tap water or 5% glucose.

4-5 DAYS AFTER BACTERIAL INOCULATION: MOUSE

No. of Bacteria Injected	WATER			5% GLUCOSE		
	Lt. Kidney	Rt. Kidney	Urine	Lt. Kidney	Rt. Kidney	Urine
10^4	10	0	0	10^5	10^4	10^4
	10	10	0	10^4	10^3	10^3
	0	10	0	10^6	10^5	10^5
	10	0	0	10^4	10^3	10^3
	10	10	10	10^5	10^5	10^5
				10^5	10^3	10^4
10^3	0	0	0	10^4	10^3	10^3
	0	0	0	10^3	10^3	10^3
	0	10	0	10^5	10^3	10^3
	0	0	0	10^3	10^5	10^4
	0	0	0	10^5	10^5	10^4
	10	0	0	10^3	10^3	10^3

TABLE III: Direct injection of E.coli into bladder of mice drinking tap water or 5% glucose. Kidneys underlined were observed to have gross abscesses.

21 DAYS AFTER BACTERIAL INOCULATION: MOUSE

WATER			5% GLUCOSE		
Lt. Kidney	Rt. Kidney	Urine	Lt. Kidney	Rt. Kidney	Urine
0	0	0	0	10^7	$>10^5$
0	0	0	10^5	10^6	$>10^5$
0	0	0	0	0	0
10^3	10^2	10	0	0	0
0	0	0	0	0	0
0	0	0	0	10^5	$>10^5$
0	0	0	10^7	10^7	$>10^5$
0	0	0	10	10^5	10^4
0	0	0	10^6	10^4	$>10^5$
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	10^5	0	10^5
0	0	0	0	0	0
0	0	0	10^4	0	10^5
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	10^5	0	$>10^5$
0	0	0	0	0	0
			0	0	0

Figure

TABLE IV

INTRAVENOUS INOCULATION OF 10^8 E. COLI
BACTERIOLOGIC STUDIES AFTER 4 DAYS

WATER			5% GLUCOSE		
Lt. Kidney	Rt. Kidney	Urine	Lt. Kidney	Rt. Kidney	Urine
0	0	0	0	0	0
10^3	0	20	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

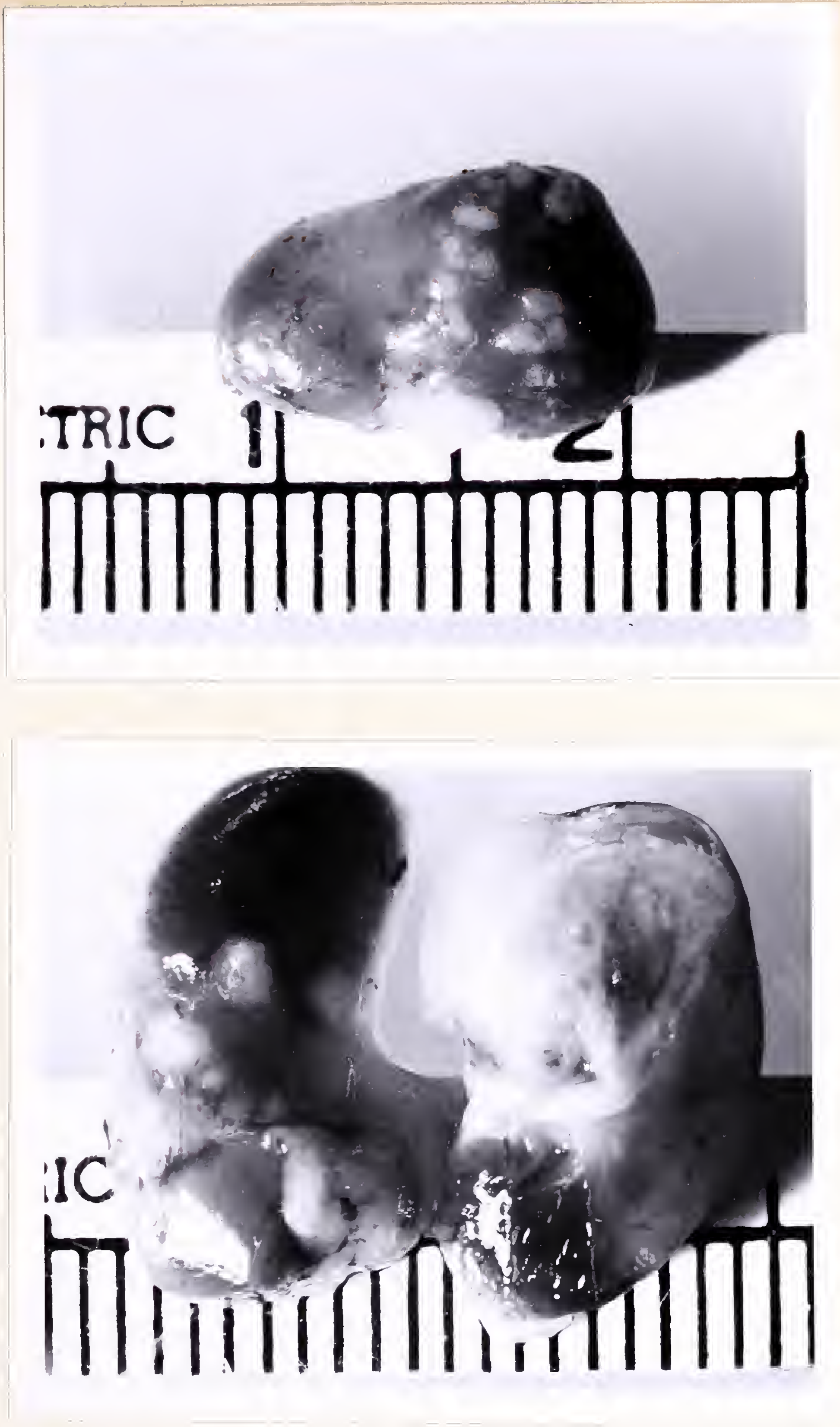


FIGURE I: Gross abscesses in two kidneys from mice with bacteriuria for 21 days while undergoing water diuresis. Scale in cm.

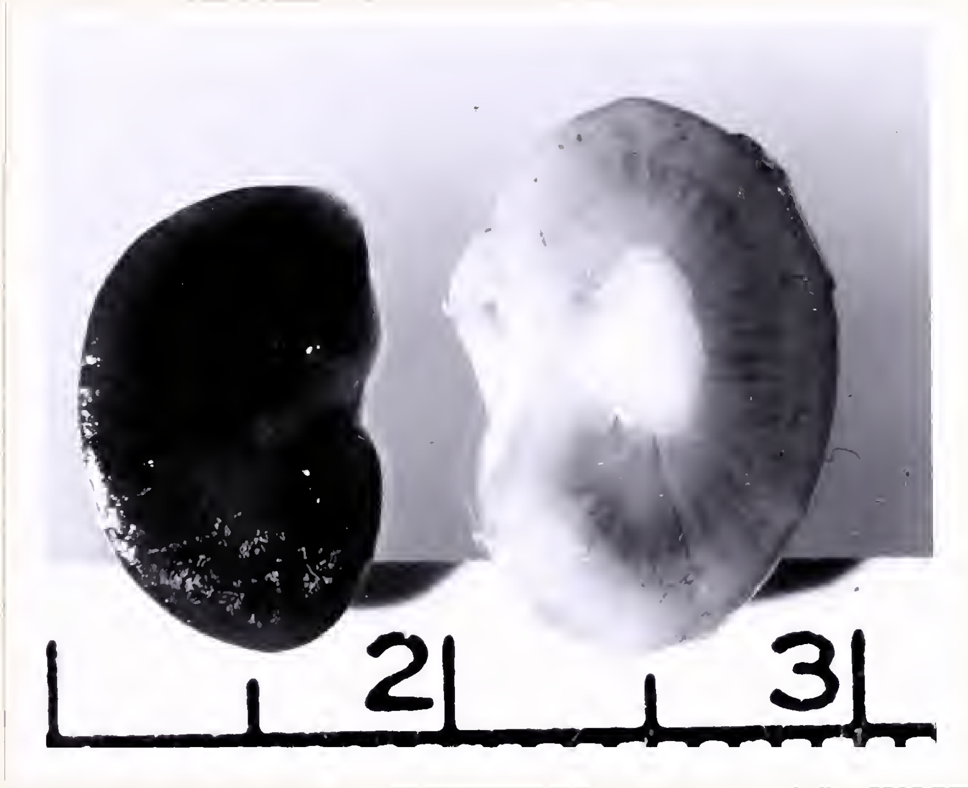
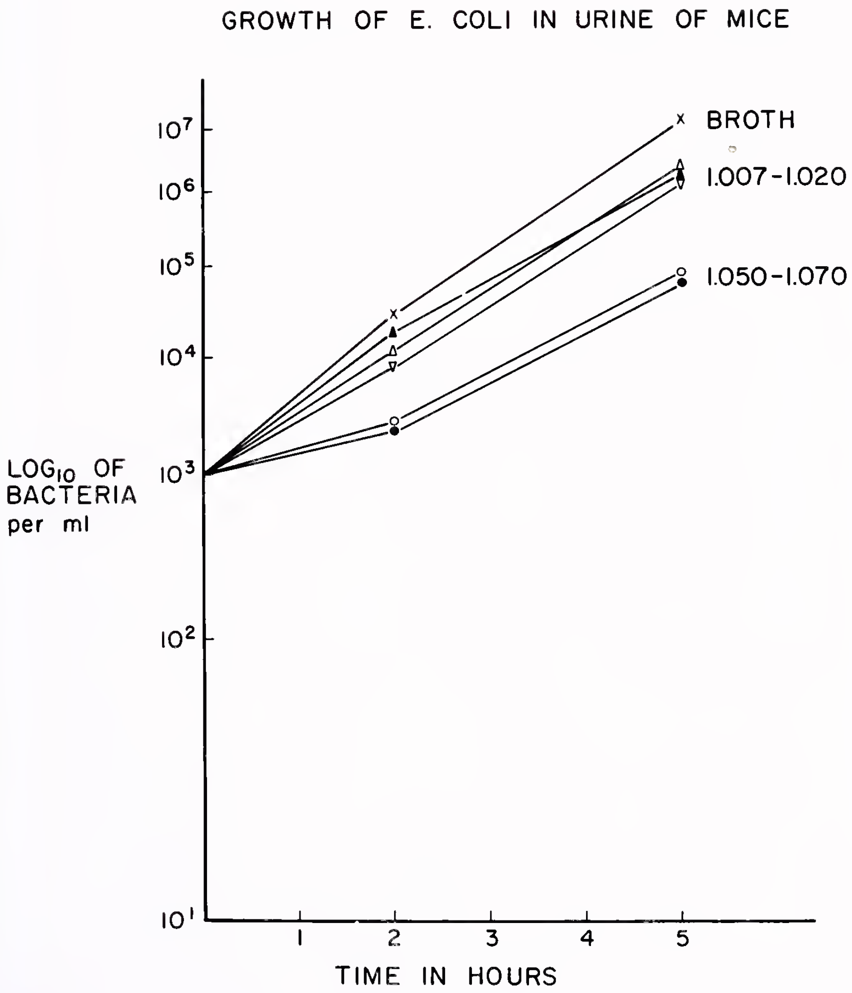


FIGURE II: Papillary necrosis in a kidney from a mouse with bacteriuria for 21 days while undergoing water diuresis. A normal kidney is shown on the left for comparison. Scale in cm.

FIGURE III


GROWTH OF E. COLI IN URINE OF MICE
LOG10 OF BACTERIA per ml
10^7
10^6
10^5
10^4
10^3
10^2
10^1
1
2
3
4
5
TIME IN HOURS
BROTH
1.007-1.020
1.050-1.070

Experimental Pyelonephritis III. Effect of water diuresis on infections produced by direct inoculation of **E. coli** into different zones of the mouse kidney.

Introduction

The preceding studies have shown that chronic water diuresis in mice induced by substituting 5% glucose for drinking water, increases susceptibility to **E. coli** urinary infection and pyelonephritis. The factors responsible for this remarkable alteration in susceptibility to infection have not been defined.

A considerable body of evidence had previously been collected which suggested that water diuresis increased resistance of the kidney to infection. Thus, it is of importance to try to determine the mechanism by which water diuresis increases susceptibility to **E. coli** infections in mice and thereby attempting a resolution of this conflict.

The present series of experiments is concerned with the effect of water diuresis on infections produced by the direct inoculation of **E. coli** into the cortex and medulla of the mouse kidney.

Results

Papillary infections during water diuresis

The direct injection of small numbers of bacteria into the renal papilla of a normal mouse results in pyelonephritis. This procedure was used in animals drinking 5% glucose to determine the effect of water diuresis on papillary infections. The results (Table I) clearly show that water diuresis was not effective in preventing pyelonephritis even when only 10 viable units were injected into the papilla. Not only was diuresis ineffectual in preventing pyelonephritis but the lesions seen often were extensive when only small number of bacteria were used in animals drinking 5% glucose (Fig. I). When 10^1-10^2 **E. coli** were injected in the papilla of mice drinking plain water, the zone of infection was small. Only following papillary inoculations with 10^4-10^5 bacteria in normal mice were large abscesses noted, comparable to that found in mice inoculated with only 10-100 viable units into the papilla under conditions of water diuresis. In addition, as has been found previously in animals inoculated with **E. coli** during water diuresis, large numbers of bacteria were routinely recovered from the opposite kidney but there was no gross evidence of infection. In contrast, bacteria were rarely recovered in large numbers from the contralateral kidney following papillary infections in mice drinking tap water. Thus, water diuresis does not prevent pyelonephritis produced by the direct injection of small numbers of **E. coli** into the renal papilla of the mouse.

Effect of trauma to the kidney during bacteriuria induced by water diuresis

When bacteria are injected onto the renal papilla, they are immediately found in the urine (12). Since **E. coli** introduced into the urinary bladder lumen during water diuresis will persist, rapidly multiply, and ascend the ureters, it was necessary to determine whether the infections seen in

animals drinking 5% glucose were a result of the direct inoculation of bacteria into the papilla or whether they were a result of the effect of water diuresis on multiplication of bacteria in the bladder urine and subsequent infection of a damaged kidney.

When 10^3-10^4 **E. coli** were directly injected into the urinary bladder of mice undergoing a water diuresis, and the renal medulla traumitized by passing a 27 gauge needle once through this region, there was no evidence of gross parenchymal infection after four days, desipte the recovery of large numbers of bacteria from the urine and both kidneys (Table II). Therefore it appears that the amount of papillary damage resulting from a single 27 gauge needle puncture does not enchance susceptability to infection during water diuresis in the mouse.

Effect of water diuresis on cortical infection

The effect of water diuresis on papillary infections and also the ability of 5% glucose to induce pyelonephritis within three weeks following injection of bacteria into the urinary bladder suggested that perhaps consumption of large amounts of carbohydrates had some as yet undetermined generalized systemic effect which increased susceptability to infection. Since water diuresis is believed to have its major effect on events within the medulla, cortical infections should not be influenced by water diuresis. In addition the effect of 5% glucose on cortical infections would be a measure of any systemic effect on susceptability to infection. The outcome of these experiments was the opposite of that seen in the medulla and in the bladder urine. Animals drinking 5% glucose were more resistant to cortical infection. When water diuresis was established with 5% glucose, infection could not be produced when as many as 10^6 **E. coli** were directly injected into the cortex of the mouse kidney. This is 1000 times the number of bacteria necessary to infect the cortex of mice drinking tap water. In addition, despite the occassional recovery of **E. coli** from the kidney and urine, there was no gross evidence of tissue infection (Table III).

When 10^7 **E. coli** were injected into the cortex of mice drinking 5% glucose, abscesses were usually found within four days (Table IV). However, the lesions seen were small and measured no more than 1-2 mm. in diameter. This was in contrast to the large abscesses seen when similar numbers of bacteria were inoculated into the cortex of mice drinking tap water. These experiments indicate that a water diuresis produce with 5% glucose has no deleterious systemic effects on susceptability to infection but rather a protective effect in the renal cortex. Thus, the substitution of 5% glucose for drinking water in the mouse increases susceptability to infection in the bladder urine and perhaps the renal papilla and decreases the susceptability in the renal cortex.

Effects of water diuresis within the cortex

Experiments were than designed to study the effect of water diuresis on inflammation and disposition of bacteria in the renal cortex. Water diuresis accelerates the inflammatory response within the medulla of the rat but not the cortex (19). Although the susceptibility of the mouse cortex to bacillary infection is different from the rat during water diuresis (30), the possibility that an accelerated cortical inflammatory response was induced in the mouse during water diuresis had to be ruled out. At intervals of 2 and 5 hours following injection of 10^3 **E.coli** into the cortex of 6 mice drinking tap water and 6 mice drinking glucose water, the kidneys were removed and serial sections made of the sites of inoculation. Careful study of these slides revealed no evidence of a significant inflammatory reaction in either group even after five hours.

The possibility that urine flow or cortical blood flow increases during water diuresis so that the inoculum is carried away from the site of injection were other factors investigated. Most experimental evidence indicates that water diuresis does not increase cortical blood flow and indeed there is some evidence suggesting that during water diuresis blood is shunted away from the cortex to the medulla (43, 44, 45). Nevertheless, experiments were designed to compare the number of bacteria recoverable 30 and 60 minutes following direct injection of bacteria into the cortex. These studies indicated that the number of organisms recovered after 1 hour from the kidney homogenates of mice drinking 5% glucose was 10-100 times lower than the number cultured from the kidneys of mice drinking tap water (Table V). The number of organisms recovered from the urine and blood of both groups was similar. Hence, the protective effect afforded to the cortex of the mouse kidney appears to be secondary to a rapid reduction in the number of bacteria inoculated. Whether this is a result of increased urine flow, increased blood flow or a combination of both could not be definitely ascertained.

Discussion

Previous experiments have demonstrated that as few as 10 **E. coli** cause pyelonephritis when directly injected into the renal papilla of normal mice. The present experiments have shown that water diuresis in normal mice induced by drinking 5% glucose does not alter the resistance of the renal medulla to pyelonephritis and indeed may increase its susceptibility to bacillary infection. On the other hand, while 10^3 **E. coli** will produce pyelonephritis in the cortex of normal mice, more than 10^6 **E. coli** are necessary to infect the renal cortex of mice undergoing a water diuresis. Thus, it appears that water diuresis not only increases susceptability of bladder urine and kidneys to **E. coli** infection but also has no protective and perhaps a deleterious effect on the host when bacteria are directly injected into the mouse kidney medulla.

Although an explanation for the inability of water diuresis to prevent or help resolve papillary infections is not at hand, data were presented demonstrating that trauma to the papilla in the presence of a chronic bacteriuria was not involved. Following direct injection of **E. coli** into the papilla, organisms can be recovered immediately from the urine. Moreover, bacteria injected into the bladder urine during a chronic water diuresis will result, within a week, in multiplication and persistance of bacteria in the urine and the recovery of large numbers of organisms from both kidneys without evidence of gross renal infection. When a 27 gauge needle was passed through the medulla and bacteria injected into the bladder urine of mice undergoing a water diuresis, there was no gross evidence of parenchymal infection despite the recovery of many organisms from both kidneys and urine. Although kidney damage has been associated with an increased susceptability to hematogenous **E. coli** pyelonephritis, the present data indicated that the damage associated with a puncture wound with a 27 gauge needle was not associated with an increased susceptability to pyelonephritis within 4 days following the production of bacteriuria during water diuresis. Hence, the inability to resolve papillary infections during water diuresis can not be explained on the basis of increased susceptability of a traumitized kidney to a chronic bacteriuria induced by a water diuresis.

The increased susceptability to urine and kidney infections of mice undergoing a water diuresis suggested that ingestion of large amounts of carbohydrate might have a systemic effect on the mouses' ability to resist infection. This seemed unlikely since Andriole and Epstein (39) have shown that rats drinking 5% glucose demonstrated an increased resistance to hematogenous staphylococcal pyelonephritis. However, the possibility that large quantities of carbohydrate might adversely effect host defense mechanisms within the mouse kidney was tested by experiments which demonstrated that water diuresis was associated with an increased resistance of the cortex to bacillary infection. Whereas 10^3 **E. coli** infected the cortex of normal mice drinking tap water, more than 10^6 organisms were needed to produce pyelonephritis in the cortex of mice drinking 5% glucose. Hence, water diuresis did not adversely effect the ability of the mouse cortex to resist infection, and therefore, a systemic effect of glucose is not a likely explanation for the increased susceptability of the mouse to urine and kidney infections during water diuresis.

Water diuresis affords quantitative protection to the mouse cortex since 10^7 **E. coli** can cause infection, albeit somewhat smaller lesions were produced. It is likely that the resistance of the cortex during water diuresis related to the rapid reduction in the number of bacteria below the critical level necessary to infect the kidney cortex.

The mechanisms by which diuresis afforded this protective effect to cortical infection was not established. When **E. coli** were directly injected into the cortex, cultures of kidney homogenates of mice drinking 5% glucose showed a signicant decrease in the number of bacteria recovered after 1 hour, whereas in mice drinking tap water the number of bacteria recovered by culture after 60 minutes was similar to the number injected.

Experiments designed to study the effect of water diuresis on the kidney indicated that water diuresis will increase urine flow and medullary blood flow, but, an increased flow of blood to the cortex seemed unlikely, and indeed, blood flow may even be slightly decreased because of shunting of blood to the medulla (43, 44, 45). However, these studies were performed in the rat and dog, not in the mouse. In addition, findings presented here indicate that there is not an acceleration of leukocyte exudation within the mouse cortex during water diuresis. Hence, phagocytosis of bacteria within the kidney cortex of mice drinking 5% glucose does not explain why bacteria are rapidly reduced in the cortex of mice undergoing a water diuresis.

The two other possibilities that were considered to explain this rapid reduction of bacteria within the cortex were an increased cortical blood flow and an increased urine flow. However, since the number of bacteria recovered from the urine of blood cultures of mice drinking tap water or 5% glucose was similar, no definite conclusions could be drawn from these findings and further investigation is necessary to define the roles, if any, of an increased urine flow or possibly increased blood flow to the cortex.

It is remarkable that in the same animal water diuresis increases the susceptibility of bladder urine and the kidney to infection yet decreases the susceptibility of the cortex to bacillary infection. This is a remarkable demonstration of the dependence of host defense mechanisms on organ architecture.

These findings are also of interest in view of the common clinical practice of forcing fluids, thereby inducing a water diuresis, in patients with urinary tract infections. There are no clinical data demonstrating the value of this long established practice in man and if the present animal studies are applicable to humans they suggest that it may be harmful to induce water diuresis in the presence of urinary tract infections.

Although the mechanisms by which water diuresis increases the susceptibility of the bladder urine and the papilla to infection and yet protects the cortex from suppcrative pyelonephritis are not completely know, the present data suggest that the hypertonic environment of the medulla is not the only and perhaps not the most important factor in explaining the peculiar susceptibility of the papilla of the kidney to infection.

Summary

In normal mice 10 **E. coli** produced infection when directly injected into the medulla, whereas 10^3 bacteria are required to infect the cortex. In mice undergoing a water diuresis (drinking 5% glucose) as few as 10 **E. coli** still produced pyelonephritis when inoculated into the renal medulla, however 10^6 organisms were needed to infect the cortex.

A chronic bacteriuria induced by water diuresis in the presence of an acutely damaged kidney was not associated with the production of pyelonephritis, indicating that this was not the mechanism by which papillary infections were produced during water diuresis.

The mechanism whereby water diuresis decreased the susceptibility of the renal cortex to infection is not clear but the data suggested that inocula were more rapidly removed from the kidney during water diuresis.

It is remarkable that water diuresis was associated with an increased susceptibility to urinary and kidney infections, and yet, in the same organ, water diuresis afforded a protective effect to cortical infections. These studies tend to exclude any systemic effect of 5% glucose on host defense mechanism as an explanation for the increased susceptibility to **E. coli** infection in the mouse during water diuresis.

APPENDIX III

Table I: Direct injection of bacteria into the renal papilla of mice drinking tap water or 5% glucose. Bacteriologic studies after 4 - 5 days. Kidneys underlined were observed to have gross abscesses.

	Tap Water			5% Glucose		
Inoculum	Lt. Kidney*	Rt. Kidney*	Urine**	Lt. Kidney*	Rt. Kidney*	Urine**
10^6	10^7	10^2	10^5	10^6	10^4	10^5
	10^7	10^2	10^6	10^5	10^5	10^5
	10^7	10^3	10^5	10^6	10^4	10^5
				10^7	10^5	10^5
				10^7	10^4	10^5
10^5	10^6	10	10^6	10^5	10^3	10^5
	10^7	10^3	10^6	10^5	10^6	10^5
	10^6	10^2	10^5	10^6	10^5	10^6
				10^6	10^5	10^5
10^4	10^6	10^2	10^5	10^5	10^5	10^6
	10^7	10^3	10^5	10^6	10^3	10^5
	10^6	10	10^4	10^5	10^4	10^4
	10^6	10	10^5	10^6	10^3	10^5
				10^7	10^5	10^5
10^3	10^5	0	10^4	10^3	10^4	10^4
	10^3	0	10^3	10^6	10^3	10^5
	10^5	10	10^4	10^5	10^6	10^6
	10^6	10^2	10^5	10^5	10^4	10^5
	10^3	0	10^2	10^4	10^4	10^5
	0	0	0			
10^2	10	0	0	10^6	10^3	10^5
	10^4	10	10^3	10^3	10^5	10^4
	0	0	0	10^6	10^4	10^5
	10^6	10	10^5	10^7	10^3	10^5
	10^5	10^2	10^4	10^4	10^5	10^5
	10^3	0	10^2	10^3	10^3	10^4
	0	0	0	10^5	10^3	10^4
10	10^2	0	10	10^5	10^3	10^4
	10^3	0	10^3	10^3	10^3	10^3
	10^5	10	10^4	10^4	10^5	10^5
	10	0	0	10^2	10^3	10^2
	0	0	0	10^5	10^4	10^5
	0	0	0			
	10^3	10^2	10^3			
	0	0	0			
	10^5	10^3	10^4			

olonies per kidney
olonies per ml.

Table II: Effect of puncture wound on kidney infections following injection of bacteria into bladder lumen of mice undergoing a water diuresis. Bacteriologic studies after 4 - 5 days. No [illegible]

Inoculum	Lt. Kidney*	Rt. Kidney*	Urine**
10^3	10^5	10^4	10^5
	10^4	10^3	10^4
	10^5	10^6	10^6
	10^5	10^6	10^6
	10^3	10^4	10^3
	10^2	10^5	10^4
	10^5	10^3	10^5
10^4	10^5	10^5	10^5
	10^6	10^5	10^6
	10^5	10^5	10^5
	10^3	10^4	10^4
	10^3	10^5	10^5

* Colonies per kidney

** Colonies per ml.

TABLE III: Direct injection of *E.coli* into renal cortex of left kidney in mice drinking tap water or 5% glucose. Kidneys underlined were observed to have gross abscesses.

CORTICAL INOCULATION OF E. COLI
BACTERIOLOGIC STUDIES AFTER 4-5 DAYS

No. of Bacteria Injected	WATER			5% GLUCOSE		
	Lt. Kidney	Rt. Kidney	Urine	Lt. Kidney	Rt. Kidney	Urine
10^3	10^5	10^2	10^4	0	0	0
	10^3	0	10^3	0	0	0
	10^3	10	10^3	0	0	0
	10^2	0	10^3	0	0	0
	10^6	10^2	$>10^5$	10^2	0	0
10^4	10^6	10^2	$>10^5$	10^2	0	0
	10^5	10	10^5	10	10	0
	10^7	10^3	$>10^5$	0	0	0
	10^6	10^2	10^4	0	0	0
	10^6	10^2	10^5	0	0	0
	10^6	10^2	$>10^5$	0	0	0
10^5	10^6	10^4	10^5	0	0	0
	10^7	10^2	$>10^5$	0	10^2	0
	10^6	10^3	$>10^5$	0	0	0
	10^7	10	$>10^5$	0	0	0
	10^6	10^2	10^5	10^2	10	0
	10^6	10	10^5	10	10	0
10^6	10^6	10^3	$>10^5$	10^2	10^2	0
	10^6	10^3	$>10^5$	10^3	10^3	10^3
	10^7	10^2	$>10^5$	10^2	0	0
	10^7	10^2	$>10^5$	10	10	0
	10^6	10^4	$>10^5$	10	0	0
	10^7	10^3	$>10^5$	10	10^2	0

Table IV: Direct injection of 10^7 *E. coli* in renal cortex of mice undergoing water diuresis. Bacteriologic studies after 4 - 5 days. Kidneys underlined were observed to have gross abscesses.

Lt. Kidney*	Rt. Kidney*	Urine*
$\underline{10^7}$	10^3	10^4
$\underline{10^6}$	10^5	10^5
10^4	10^3	10^4
$\underline{10^6}$	10^4	10^5
$\underline{10^6}$	10^6	10^5
$\underline{10^5}$	10^5	10^5
$\underline{10^6}$	10^5	10^6
$\underline{10^5}$	$\underline{10^5}$	10^5
10^4	10^6	10^6

* Colonies per kidney

** Colonies per ml.

Table V: Direct injection of bacteria into the left cortex of mice drinking tap water of 5% glucose. Bacteriologic studies after 30 and 60 minutes.

	Tap Water			5% Glucose		
Inoculum 30 Minutes	Lt. Kidney*	Rt. Kidney*	Urine**	Lt. Kidney*	Rt. Kidney*	Urine**
10^7	1×10^7	10^3	10^2	6×10^6	10^4	50
	7×10^6	10^4	10^4	7×10^6	10^4	10^2
	1×10^7	10^5	10^2	3×10^6	10^4	10^3
10^6	2×10^6	10^4	10^2	2×10^5	10^4	10^3
	4×10^6	10^3	0	9×10^5	10^3	0
	4×10^6	10^3	10^2	2×10^5	10^4	10^3
	6×10^6	10^3	10^2	1×10^6	10^3	10^2
10^5	1×10^5	0	0	6.7×10^4	10^2	60
	1×10^5	10^2	30	6.1×10^4	0	0
	1×10^5	10	0	1.0×10^5	10^2	0
	2×10^5	0	0	5×10^4	0	0
60 Minutes						
10^7	1×10^7	10^5	10^3	4×10^6	10^4	10^4
	7×10^6	10^3	10^3	1×10^6	10^4	10^2
10^6	5×10^6	10^4	10^3	9×10^5	10^3	20
	8×10^6	10^3	10^2	1×10^5	10^3	10^3
	7×10^6	10^3	10^2	2×10^5	10^4	10^3
	7×10^6	10^4	10^3	3×10^5	10^4	10^3
10^5	9×10^5	10^3	10	7.8×10^4	10^3	10^2
	1×10^5	10^3	10^2	9×10^3	10^2	10
	2×10^5	10^3	10	1.7×10^4	10^2	10^2
	2×10^5	10^2	10	1×10^4	10^2	10^3

* Colonies per kidney.

** Colonies per ml.

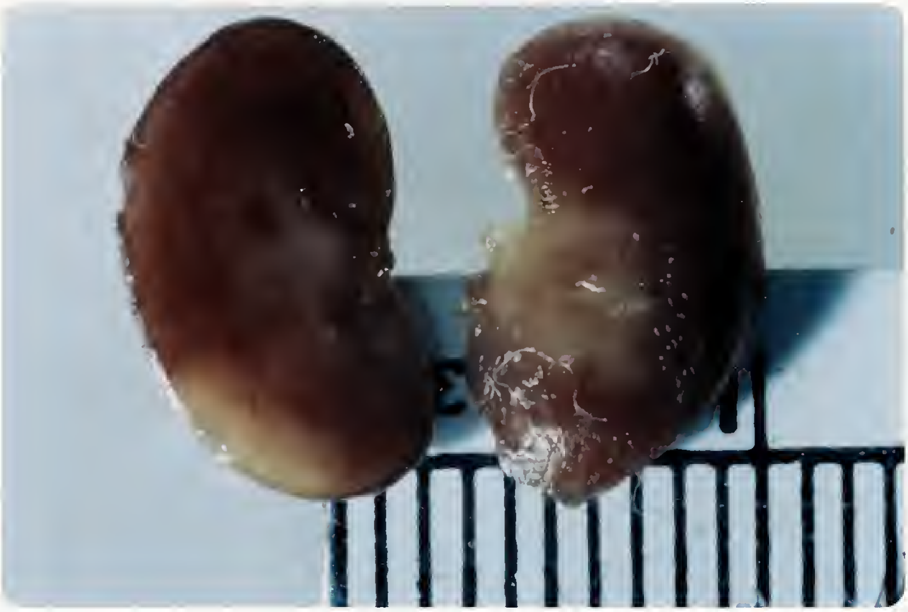


FIGURE I: Gross abscess in kidney produced by direct injection of 10^2 *E.coli* into papilla during water diuresis. Scale in cm.

Conclusion

1. The present experiments demonstrates that the cortex and medulla of the mouse kidney signifacantly differed in their ability to resist infection when either staphylococci or **E. coli** are directly injected into these regions. Whereas 10^3 bacteria produced pyelonephritis in the cortex fewer than 10 organisms infected the medulla. These experiments confirmed earlier studies in the rabbit.

2. The evidence presented indicates that neither serum bactericidal activity nor kidney lysozyme played significant roles in the pathogenesis of these infections.

3. Water diuresis induced by drinking 5% glucose was not effective in preventing papillary infection and based on gross evidence, appeared to make them worse. In contrast, water diuresis prevented cortical infections when as many as 10^8 **E. coli** were directly injected into this region of the kidney.

4. When **E. coli** were injected into the bladder lumen during a water diuresis, bacteria persisted, multiplied and were recoverable from both kidneys after four days. In contrast, if the mice were drinking tap water the bacteria were rapidly and efficiently cleared from the bladder urine.

5. A chronic bacteriuria with **E. coli** induced by water diuresis resulted in severe pyelonephritis which was at times bilateral and demonstrated papillary necrosis.

6. These experiments indicate that a water diuresis increased the susceptibility of the mouse urine and kidney to infection. However, this increased susceptibility to infection was not related to the ingestion of large amounts of carbohydrate since drinking 5% glucose was associated with an increased re-of the cortex to pyelonephritis. There was no glycosuria and blood sugar were not elevated in mice drinking 5% glucose.

7. The induction of a chronic bacteriuria during water diuresis has clinical implications. Forcing fluids has long been considered beneficial in urinary tract infection in man. However, this impression is not based on any studies comparing the outcome of therapy in patients undergoing water diuresis or in patients on normal fluid intake. The present studies, if applicable to man, emphasized the dangers that may be associated with this procedure. Furthermore, these experiments provided an animal model that may be employed to investigate the effects of chronic bacteriuria on such factors as blood pressure and pregnancy.

8. Finally this model of chronic bacteriuria induced by water diuresis may be a clue to increased susceptibility to pyelonephritis in clinical states associated with increased rates of urine flow and concentrating defects such as renal insufficiency, diabetes mellitus, potassium depletion, diabetes insipidus, diuretic phase of acute tubular necrosis and a post obstructive diuresis.

References

1. Guze, L. B. and Beeson, P. B.: Experimental Pyelonephritis. I. Effect of ureteral ligation on the course of bacterial infection in the kidney of the rat. J. Exp, Med., 1956, **104**, 803.

2. Smith, J. M. and Dubas, R. J.: The behavior of virulent and avirulent staphylococci in the tissues of normal mice. J. Exp. Med., 1956, **103**, 87.

3. Lippman, M.: Thesis, Yale University School of Medicine. 1968, In preparation.

4. Cohn, Z. A. and Hirsch, J. G.: The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. J. Exp. Med., 1960, **112**, 983.

5. Osserman, E. F. and Lawlor, D. P.: Serum and urinary lysozyme (muramidase) in monocytic and myelomonocytic leukemia. J. Exp. Med., 1966, **124**, 921.

6. De Navasquez, S.: Experimental pyelonephritis in the rabbit produced by staphylococcal infection. J. Path. Bact., 1950, **62**, 429.

7. Gray, J. E., Wilkins, J. R., Prestrud, M.C. and Nukitas, C.T. Further characterization of an experimental staphylococcal infection in mice. J. Infect. Dis., 1957, **101**, 137.

8. Gorrill, R. H.: The establishment of staphylococcal abscesses in the mouse kidney. Brit. J. Exp. Path., 1958, **39**, 203.

9. Freedman, L. R., Werner, A.S., Beck, D., and Paplanus, S.: Experimental pyelonephritis IX. The bacteriological course and morphological consequences of staphylococcal pyelonephritis in the rat, with consideration of the specificity of the pathological changes observed. Yale J. Biol. Med., 1961, **34**, 40.

10. De Navasquez, S.: Futher studies in experimental pyelonephritis produced by various bacteria, with special reference to renal scarring as a factor in pathogenesis. J. Path and Bact., 71: **27**, 1956.

11. Rocha, H. Guze, L., Freedman, L. L, Beeson, P. B.: Experimental pyelonephritis. III. The influence of localized injury to different parts of the kidney on susceptibility to bacillary infection. Yale J. Biol., 1958, **30**, 341.

12. Freedman, L. R. and Beeson, P. B.: Experimental pyelonephritis IV. Observations on infections resulting from direct inoculation of bacteria into different zones of the kidney. Yale J. Biol. Med., 1958, **30**, 406.

13. Freedman, L. R.: Experimental pyelonephritis. VI. Observations on susceptibility of the rabbit kidney to infection by a virulent strain of Staphylococcus Aureus. Yale J. Biol. Med., 1960, **32**, 272.

14. Gorrill, R. S. and DeNavasquez, S.: The pathogenesis and evolution of experimental pyelonephritis in the mouse with special reference to comparable conditions in man. J. Path. Bact., 1960, **80**, 239.

15. MacLeod, C. M., Hall, C. A. and Frohman, L. A.: Relationship of abscess formation in mice, guinea pigs and rabbits to antistaphlococcal activity of their tissues and blood serum. Brit. J. Exp. Path., 1963, **44**, 612.

16. Florey, H.: The relative amounts of lysozyme present in the tissues of some mammals. Brit. J. Exp. Path., 1930, **11**, 251.

17. Spuce, A. J.: Histo chemical distribution of lysozyme activity in organs of normal mice and radiation chimeros. J. Histochem. Cyteehm., 1964, **12**, 384.

18. Glynn, A. A. and Parkman, R.: Studies with an antibody to rat lysozyme. Immunology, 1964, **7**, 724.

19. Andriole, V. T.: Acceleration of the inflammatory response of the renal medulla by water diuresis. J. Clin, Invest., 1966, **45**, 847.

20. Rocha, H. and Fekety, F.: Acute inflammation in the renal cortex and medulla following thermal injury. J. Exp. Med., 1964, **119**, 131.

21. Cohn, Z. A. and Morse, S. I.: Interaetions between rabbit polymorphonuclear leucocytes and staphylococci. J. Exp. Med., 1959, **100**, 419.

22. Beeson, P. B. and Rowley, D.: The anticomplementary effect of kidney tissue, its association with ammonia production. J. Exp. Med., 1959, **110**, 685.

23. Salton, M. R. J.: The lysis of micro-organisms by lysozyme and related enzymes. J. Gen. Microbiol., 1958, **18**, 481.

24. Keane, W. and Lippman, M.: Unpublished data.

25. Cappuccino. J. Winston, S, and Perri, G.: Muramidase activity of kidney and spleen in Swiss mice challenged with BCG, zymosan and bacterial endotoxins. Proc. So. Exp. Biol. Med., 1964, **116**, 869-872.

26. Keane, W. F. and Freedman, L. R.: **E. coli** pyelonephritis in mice during water diuresis. Clin. Research, 1967, **15**, 468.

27. Vivaldi, E., Cotran, R., Zangivill, D. P. and Kass, E.: Ascending infection as a mechanism in the pathogenesis of experimental nonobstructive pyelonephritis. Proc. Soc. Exp. Biol. (N.Y.), 1959, **102**, 242.

28. Freedman, L. R.,: Pyelonephritis and urinary tract infection. In: Diseases of the kidney, Ed. by M. B. Strauss and L. G. Welt, Boston, Little, Brown, and Co. 1963, pp. 469.

29. Beeson, P. B.: Factors in the pathogenesis of Pyelonephritis. Yale J. Biol. Med., 1955, **28**, 81.

30. Freedman, L.R.: Experimental pyelonephritis, XIII. On the ability of water diuresis to induce susceptibility to **E. coli** bacteriuria in the normal rat. Yale J. Biol. Med., 1967, **39**, 255.

31. Cox, C.E., and Hinman, F., Jr.; Experiments with induced bateriuria vesical emptying and bacterial growth on the mechanism of bladder defense to infection. J. Urol., 1961, **86**, 739.

32. Carone, F. A. Fashgarian, M., Epstein, F.H.,: Effect of acute potassium deficiency on susceptibility to infection with particular reference to the kidney. Yale J. Biol. Med., 1959, **32**, 100.

33. Wood, W. B., Jr.: Studies on the cellular immunology of acute bacterial infections, Harvey Lectures, series 47, 72-98, 1951-52.

34. Mehrota, R.M.L.: An experimental study of the vesical circulation during distention and in cystitis. J. Path. Bact., 1953, **66**, 79.

35. Sommer, J.L.: Experimental pyelonephritis in the rat with observations on ureteral reflux. J. Urol., 1961, **86**, 375.

36. Anderson, B.R. and Jackson, G.G.: Pyelitis an important factor in the pathogenesis of retrograde pyelonephritis. J. Exp. Med., 1961, **114**, 375.

37. Heptinstall, R.H.: Bacteriological and morphological studies on the ascending route of infection in the rat. Nephron, 1964, **1**, 73.

38. Heptinstall, R.H.: Experimental pyelonephritis. Ascending infection of the rat kidney by organisms residing in the urethra. Brit. J. Exp. Path., 1964, **45**, 436.

39. Andriole, V.T. and Epstein, F.H.: Prevention of pyelonephritis by water diuresis: Evidence for the role of medullary hypertonicity in promoting renal infection. J. Clin, Invest., 1965, **44**, 73.

40. Smith, B.N.: Fluid intake and experimental pyelonephritis, In: Progress in pyelonephritis, Ed. E, H. Kass, Philadelphia, F.A. Davis, Co. pp. 239-241, 1965.

41. Mahoney, S.A. and Persky, L.: Observations on experimental ascending pyelonephritis in the rat. J. Urol, 1963, **89**, 779.

42. Kass, E.H.: The role of asymptomatic bacteriuria in the pathogenesis of pyelonephritis, in Biology of pyelonephritis (Henry Ford International Symposium) E. by E. H. Kass. Little, Brown and Co. (Boston) 1960 pp. 399-412.

43. Ullrich, K. J., Kramer, K. and Boylan J.W.: Present knowledge of the counter-current system in the mammalian kidney. Prog. Cardiovasc. Disc., 1961, **3**, 395.

44. Thurau, K., Deetjen, P. and Kramer, K.: Hamodynamik des Nierenmarks. II, Mitteilung. Wechaselbeziehung zwischen bascularem imd tubularem Gegenstromsystem bei arteriellen Drucksteigerungen, Wasserdiuresis Und osmotischer Diuresis. Pflugers Arch. Ges. Physiol., 1960, **270**, 270.

45. Earley, L. and Friedeer, R.M.: Changes in renal blood flow and possibly the intrarenal distribution of blood accompanying the Natriuresis of Saline loading. J. Clin. Invest., 1965, **44**, 1042.

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